

In re Appln. of HISADA et al.
Application No. 09/622,206

CLAIM AMENDMENTS

1. (Currently Amended) A method for quantitatively detecting an antigen which comprises:

a first step of providing an Fab' antibody having a uniform isoelectric point, said antibody forming an immune complex with an antigen in an analytical sample and being modified by adding an amino acid sequence comprising a charged amino acid residue and by being labeled with a fluorescent dye;

a second step of mixing the Fab' antibody having a uniform isoelectric point with the analytical sample containing the antigen to obtain a mixture comprising the immune complex;

a third step of separating the mixture by performing electrophoresis in a carrier;

a fourth step of irradiating an excitation light which excites the fluorescent dye to the mixture separated in the third step to cause fluorescence in the immune complex; and

a fifth step of detecting the ~~fluorescence~~ fluorescence and correlating the detected fluorescence with the amount of antigen;

wherein the amino acid sequence is added adjacent to a C-terminal of an L chain of the Fab' antibody having a uniform isoelectric point.

2. (Canceled)

3 (Previously Presented) The method according to claim 1, wherein the fluorescent dye is bound to a cysteine residue which is not involved in binding with an L chain and which exists in an amino acid sequence adjacent to a C-terminal of a CH1 region of the Fab' antibody having a uniform isoelectric point.

4. (Previously Presented) The method according to claim 1, wherein the electrophoresis is performed by isoelectric focusing.

5. (Previously Presented) The method according to claim 1, wherein the electrophoresis is performed by capillary electrophoresis.

6.-7. (Canceled)

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8. (Previously Presented) The method according to claim 1, wherein the Fab' antibody having a uniform isoelectric point is produced by a method which comprises:

- a first step of providing an Fd chain gene encoding a VH region and CH1 region, and an amino acid sequence which adjoins to a C-terminal of the CH1 region and comprises a cysteine residue which is not involved in binding with an L chain in an Fab' antibody, and an L chain gene encoding the L chain of the Fab' antibody;
- a second step of linking the Fd chain gene and the L chain gene in the expressible state to obtain a gene expressing an Fab' antibody;
- a third step of modifying the gene expressing an Fab' antibody to express an amino acid sequence comprising a charged amino acid residue adjacent to a C-terminal of the L chain, and site-specifically mutating in the gene expressing an Fab' antibody at least one codon encoding an amide group-containing amino acid residue in the CH1 region, into a codon encoding an amide group-non-containing amino acid residue except for cysteine to obtain a gene expressing a charge modified Fab' antibody;
- a fourth step of transforming a host cell with the gene expressing a charge modified Fab' antibody and culturing the resultant transformant to obtain an Fab' antibody having a uniform isoelectric point, the Fab' antibody being modified by adding an amino acid sequence comprising a charged amino acid sequence comprising a cysteine residue which is not involved in binding with an L chain adjacent to the C-terminal CH1 region, and
- a fifth step of binding a fluorescent dye to the cysteine residue which is not involved in binding with an L chain in the Fab' antibody having a uniform isoelectric point obtained in the fourth step.

9.-21. (Canceled)